REMARKS

I. OVERVIEW

Claims 14-15, 20-21, and 24 are now pending in this application. Claims 15, 20, 21, and 24 have been canceled. Claim 14 has been amended. Claims 30-33 have been added. Support for these amendments can be found in the specification at pages 7-9 and also in Figure 2. No new matter has been added. The present response is an earnest effort to place all claims in proper form for immediate allowance. Reconsideration and passage to issuance is, therefore, respectfully requested.

II. CLAIM REJECTIONS 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 14 and 20 stand rejected under 35 U.S.C. § 112, second paragraph for being indefinite. The Examiner states that although Applicants state in their response that claim 14 has been amended to recite "said protease" said amendments have not been made. Therefore, the Examiner maintains the rejection.

Applicants thank the Examiner for pointing out this inadvertent mistake and Applicants have appropriately amended claim 14 so that it now recites "A method of assaying for protease activity inside a cell, comprising: measuring an initial fluorescence activity in said cell to establish a baseline; introducing into said cell a nucleic acid construct having a sequence encoding a chimeric protein comprising an amino terminal portion of a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; wherein the presence of a peptide bond between an amino terminal portion and a carboxyl-terminal portion of said protease substrate sequence is essential to generate or maintain fluorescence of said chimeric

protein; and measuring a change in the fluorescence activity caused by proteolytic cleavage of said chimeric protein in said cell."

Claim 20 has been canceled rendering this rejection moot. Therefore, Applicants request that the rejection under 35 U.S.C. § 112, second paragraph be withdrawn and claim 14 be reconsidered.

III. CLAIM REJECTIONS 35 U.S.C. § 103

Claims 14 and 15 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Mahajan et al, 1999 in view of Abedi et al, 1998, for the reasons explained in the First Action on the Merits, mailed September 23, 2003, and the Final Rejection, mailed March 10, 2004.

Claims 20, 21 and 24 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Mahajan et al, 1999, in view of Abedi et al, 1998, and further in view of Martin et al., 1997 for the reasons described in the Final Rejection. These rejections will be traversed collectively.

Applicant respectfully submits that the Office Action did not make out a *prima facie* case of obviousness for the following reasons: the fact that references can be combined or modified is not sufficient; and there is no suggestion to combine the cited references because a suggestion to combine must come from the prior art and not from Applicant's specification or impermissible hindsight.

Examiner cites *Ruiz v A.B. Chance Co.*, 357 F.3d 1270, 1276, 69 USPQ2d 1686, 1690 (Fed. Cir. 2004) for the proposition that finding of obviousness does not require existence of express, written motivation to combine in prior art. At the same time, however, although it is not necessary that the cited references or prior art specifically suggest making the combination, there must be some teaching somewhere which provides the suggestion or motivation to combine prior

art teachings and applies that combination to solve the same or similar problem which the claimed invention addresses. One of ordinary skill in the art will be presumed to know of any such teaching. (See, e.g., *In re Nilssen*, 851 F.2d 1401, 1403, 7 USPQ2d 1500, 1502 (Fed. Cir. 1988) and *In re Wood*, 599 F.2d 1032, 1037, 202 USPQ 171, 174 (CCPA 1979)).

One of skill in the art would not be motivated to combine Mahajan with Abedi. The nature of Applicants' problem to be solved would not lead one of skill in the art to combine these references for possible solutions to that problem. Applicants' invention relates to assaying for protease activity inside said cell, comprising: measuring an initial fluorescence activity in said cell to establish a baseline; introducing into a cell a nucleic acid construct having a sequence encoding a chimeric protein comprising an amino terminal portion of a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; wherein the presence of a peptide bond between an amino terminal portion and a carboxyl-terminal portion of said protease substrate sequence is essential to generate or maintain fluorescence of said chimeric protein; and measuring a change in the fluorescence activity caused by proteolytic cleavage of said chimeric protein in said cell.

Mahajan describes treating cells with an apoptotic agent, where a cell contains a vector expressing two different fluorescent proteins joined by a peptide bridge containing a potential caspase-cleavage site. Mahajan et al., at page 408. It is to be noted that Examiner seems to be taking a very broad view of the disclosure in Abedi. Examiner states that Abedi teaches a GFP as a scaffold for peptide *substrates* inserted within the GFP sequence. Office Action, at page 3. (emphasis added). It is submitted that this disclosure is much broader than the actual disclosure in Abedi. Substrates, as defined, are specific molecules upon which enzymes act. Abedi never

describes nor even suggests the use of peptides as *substrates* in his library. Rather, Abedi only relates to the production of a peptide-*aptamer* library where peptide aptamers are short oligopeptides designed to bind tightly to their targets with high affinity, i.e. low dissociation constants, as the oligopeptides are displayed from a scaffold protein. Abedi et al., Abstract, at page 623. The peptide aptamer-library reported by Abedi was undertaken to develop a new method for ligand discovery and states that the impetus for new experiments such as his is the "desire to either isolate peptide-based inhibitors of particular targets or establish the normal binding partners of known proteins in the cell." Abedi et al., at page 623, column 1, 1st paragraph. There is no suggestion that this teaching should be applied to assaying for proteolytic cleavage of a substrate and, furthermore, Abedi has an entirely different purpose than present inventors.

The present invention's problem to be solved of assaying for proteolytic cleavage of a substrate does not involve ligand discovery, using aptamers as inhibitors or binding partners as described in Abedi. The present invention is not assaying for potential inhibitors of a particular target protein, nor is it assaying for a potential binding partners of a particular target protein. Rather, a method of the present invention involves assaying for proteolytic cleavage of a chimeric protein containing a substrate sequence and a green fluorescent protein sequence. Therefore, one skilled in the art would not look to a paper creating a new tool for discovering protein inhibitors or binding partners when trying to determine a way to profile proteolytic cleavage of a substrate.

Neither Mahajan nor Abedi suggest the desirability of combining assaying for protease activity inside a cell, comprising: measuring an initial fluorescence activity in said cell to establish a baseline; introducing into said cell a nucleic acid construct having a sequence

encoding a chimeric protein comprising an amino terminal portion of a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; wherein the presence of a peptide bond between an amino terminal portion and a carboxyl-terminal portion of said protease substrate sequence is essential to generate or maintain fluorescence of said chimeric protein; and measuring a change in the fluorescence activity caused by proteolytic cleavage of said chimeric protein in said cell.

One of skill in the art would not combine Mahajan with Abedi since Abedi is directed towards isolating peptide-based inhibitors of particular targets or establishing a protein's normal binding partner(s). There is no teaching in Mahajan or Abedi that provides the suggestion or motivation to combine prior art teachings and applies that combination to solve the same or similar problem of the instant invention of assaying for proteolytic cleavage of a substrate. Therefore, the claimed invention is inventive and not obvious.

One of skill in the art would not be motivated to combine Mahajan with Abedi and with Martin. The nature of Applicants' problem to be solved would not lead one of skill in the art to combine these references for possible solutions to that problem. Applicants' invention is assaying for protease activity inside a cell, comprising: measuring an initial fluorescence activity in said cell to establish a baseline; introducing into said cell a nucleic acid construct having a sequence encoding a chimeric protein comprising an amino terminal portion of a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; wherein the presence of a peptide bond between an amino terminal portion and a carboxyl-terminal portion of said protease substrate sequence is essential to generate or maintain

fluorescence of said chimeric protein; and measuring a change in the fluorescence activity caused by proteolytic cleavage of said chimeric protein in said cell.

The teachings and problems to be solved by Mahajan and Abedi are previously described. The Martin reference is directed towards finding an inhibitor of HCV NS3 protease. More specifically, the Martin reference describes "small protein scaffolds on which functions can be engineered ... as a means of generating conformationally defined structures with potential as pharmacophores." Martin, at page 607, 2nd paragraph. Martin is directed towards finding a NS3 protease inhibitor based on the three-dimensional arrangement of functional groups required for a molecule to exert a particular biological effect, here, "small inhibitors of its [HCV NS3] enzymatic activity." Martin, at page 608, 1st paragraph. Martin uses aptamers for the selection of NS3 inhibitors based on binding affinity for the protease, for example, at its active site cleft. Martin, at page 613, 2nd column, 2nd paragraph. In contrast, the present invention is directed towards assaying for proteolytic activity of proteases on substrates, not using peptides to bind with high affinity to a protease, thereby inhibiting enzymatic activity. Nor is the present invention directed towards designing peptides based on their three-dimensional arrangement as Martin teaches.

Neither Mahajan nor Abedi nor Martin suggest the desirability of combining assaying for protease activity inside a cell, comprising: measuring an initial fluorescence activity in said cell to establish a baseline; introducing into said cell a nucleic acid construct having a sequence encoding a chimeric protein comprising an amino terminal portion of a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; wherein the presence of a peptide bond between an amino terminal portion and a carboxyl-terminal portion of

said protease substrate sequence is essential to generate or maintain fluorescence of said chimeric protein; and measuring a change in the fluorescence activity caused by proteolytic cleavage of said chimeric protein in said cell.

One of skill in the art would not be motivated to combine Mahajan with Abedi and with Martin since Abedi is directed towards isolating peptide-based inhibitors of particular targets or establishing a protein's normal binding partner(s). Martin is directed towards inhibiting a viral protease using aptamers for the selection of NS3 inhibitors based on binding affinity for the protease. There is no teaching in Mahajan or Abedi or Martin that provides the suggestion or motivation to combine prior art teachings and applies that combination to solve the same or similar problem of the instant invention of assaying for proteolytic cleavage of a substrate. Thus, the claimed invention is clearly inventive over the prior art of Mahajan in view of Abedi and further in view of Martin. Therefore, the claims are inventive and not obvious. Applicants request that the rejections be withdrawn and reconsidered.

IV. NEW ISSUES

A. Specification-Objections

The Examiner notes that on page 5, line 13, "bacterial" should be corrected to "bacteria". Applicants thank Examiner for pointing out this inadvertent grammatical error and have accordingly amended the specification so that it now recites "substrate co-transformed into bacteria with the protease".

B. Oath-Objections

The Examiner states that the Oath of April 18, 2002 is objected to for having incomplete information regarding the claim to priority. The Examiner states that the claim to priority recites only that the priority application is a PCT and that the date of filing is February 8, 2002. The

Examiner states that the Oath fails to provide the serial number or country code for said PCT application. Applicants submitted an oath on February 8, 2002 for the instant application which in the first paragraph claims priority to Provisional Application Serial No.: 60/267,440. Therefore, Applicants request that this objection be withdrawn.

C. Claims-Objections

The Examiner objects to claim 14 for grammatical reasons. The Examiner states that the phrase "linked to a serine protease substrate sequence" should be amended to "link to a sequence encoding a serine protease substrate sequence".

Applicants would like to thank the Examiner for pointing out this inadvertent error and accordingly have amended claim 14 so that it now recites "a green fluorescent reporter protein operably linked to a sequence encoding a protease substrate sequence". Therefore, Applicants request that the objection to claim 14 be withdrawn.

The Examiner objects to claim 20 for grammatical sentence construction and states that the phrase "linked to a NS3/4A serine protease substrate sequence that encodes a serine protease substrate" should be amended to "linked to a sequence encoding a NS3/4A serine protease substrate sequence". Claim 20 has now been canceled rendering this objection moot. Therefore, Applicants request that the objection to claim 20 be withdrawn.

V. CLAIM REJECTIONS 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 14, 15, 20 and 21 stand rejected under 35 U.S.C. § 112, second paragraph, for improper antecedent usage. The Examiner states that claims 14 and 20 each recite the limitation "the serine protease substrate sequence" and states that there is insufficient antecedent basis for this limitation in the claims because what is being expressed is not just the serine protease

substrate but the nucleic acid construct encoding a fluorescent fusion protein comprising the serine protease substrate sequence. Claim 20 has been canceled rendering this objection moot. Accordingly, claim14 has been amended so that they now recite "a protease substrate sequence".

Claim 14 stands rejected because the phrase "protease activity" fails to provide a correct antecedent basis. The Examiner suggests that the phrase "protease activity" be amended to "said protease activity". Claim 14 has been amended so that it no longer recites "protease activity" rendering this objection as moot.

Claims 15 and 21 stand rejected under 35 U.S.C. § 112, second paragraph, because they recite the limitation "the amino and carboxyl-terminal fragment of . . . ". The Examiner states that there is insufficient antecedent basis for this limitation. Claims 15 and 21 have been canceled, rendering this rejection moot. Therefore, Applicants request that the rejection to claims 15 and 21 under 35 U.S.C. § 112, second paragraph, be withdrawn.

Claim 20 stands rejected under 35 U.S.C. § 112, second paragraph, because claim 20 recites the limitation "the serine protease substrate sequence" and lacks sufficient antecedent basis. The Examiner states that what is being expressed is not just the serine protease substrate sequence, but also the nucleic acid construct encoding a fluorescent fusion protein comprising a serine protease substrate. Claim 20 has been canceled rendering this rejection moot. Therefore, Applicants request that the rejection to claim 20 under 35 U.S.C. § 112, second paragraph, be withdrawn and reconsidered.

Claim 21 stands rejected under 35 U.S.C. § 112, second paragraph, because claim 21 recites the limitation "the serine protease substrate" and lacks sufficient antecedent basis. The Examiner suggests that claim 21 be amended so it recites "the serine protease substrate sequence". Claim 21 has been canceled rendering this rejection moot. Accordingly, Applicants

request that the rejection to claim 21 under 35 U.S.C. § 112, second paragraph, be withdrawn and reconsidered.

CONCLUSION

This is a request to extend the period for filing a response in the above-identified application for one month from February 2, 2005 to March 2, 2005. Applicant is a small entity; therefore, please charge Deposit Account number 26-0084 in the amount of $60.00 to cover the cost of the one month extension. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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